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APIUMOSIDE, A NEW FURANOCOUMARIN GLUCOSIDE FROM THE SEEDS OF *APIUM GRAVEOLENS*

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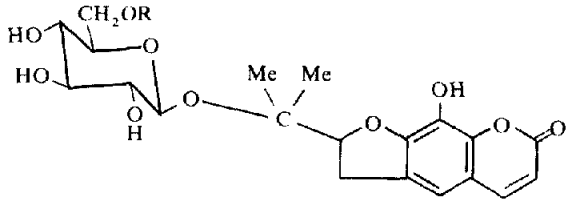
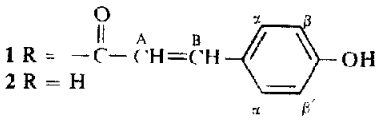
Key Word Index—*Apium graveolens*; Umbelliferae: apiumoside; coumarin glucoside; structural determination.

Continuing our investigation [1, 2] of *Apium graveolens* seeds, we now report the isolation of a new coumarin glucoside, apiumoside (**1**), from the ethyl acetate extract of the seeds.





1 R =
2 R = H

The glycoside, **1**, mp >300° was obtained as a pale yellow glassy solid. The 90 MHz ^{1}H NMR spectrum of the glycoside acetate showed the presence of rutaretin [1, 3] and *p*-substituted styryl units in **1**. Moreover, the presence of only three aliphatic acetoxyls indicated that two sugar hydroxyls are involved in the linkage. Acid hydrolysis of **1** gave rutaretin [1], *p*-coumaric acid and D-glucose. Formation of 9-*O*-methyl rutaretin [1] and *p*-methoxycinnamic acid on methylation (Me_2SO_4) of **1**, followed by hydrolysis, showed the involvement of tertiary hydroxyl of rutaretin with the sugar unit. That the acid unit is ester-linked to glucose was confirmed by the graded hydrolysis of **1** to give rutaretin-1′-*O*-glucoside (**2**) and *p*-coumaric acid. The linkage of glucose to rutaretin through the C-1 hydroxyl was shown by permethylation of **2** and subsequent hydrolysis of the permethylate [4] yielding 2, 3, 4, 6-tetra-*O*-methyl-D-glucopyranose, which was confirmed by PC. Further, the formation of 2,3,4-tri-*O*-methyl-D-glucopyranose on similar treatment of **1** established the (1 → 6) linkage of glucose with rutaretin and the acid, respectively. Finally, β-linkage of the glucose was confirmed by enzymatic hydrolysis. Thus **1** was identified as (−)-2,3-dihydro-9-hydroxy-2 [1-(6-*p*-coumaroyl) β-D-glucosyloxy-1-methyl ethyl]-7H-furo-[3,2g][1]-benzopyran-7-one.

EXPERIMENTAL

Isolation of glucoside. Dried *Apium graveolens* seeds (4.0 kg) were extracted successively with petrol, C_6H_6, Et_2O and EtOAc. The EtOAc extract was coned and chromatographed on Si gel (500 g) with $CHCl_3$ → MeOH gradient. The fractions eluted with $CHCl_3$–MeOH (23:2) on PLC (Si gel; EtOAc–MeOH–H_2O, 100:16.5:13.5) afforded the glucoside (**1**).

Identification. **1** was obtained as a pale yellow glassy solid (500 mg), mp>300°; $[\alpha]_D^{25}$ −37.37° (*c* 0.550, MeOH); R_f: 0.60 (EtOAc–MeOH–H_2O, 100:16.5:13.5); 0.45 ($CHCl_3$–MeOH, 3:1); UV λ_{max} nm (log ε): 265 (4.04), 320 (4.48); IR ν_{max}^{KBr} cm^{-1}: 3450, 1680, 1625, 1500, 1267 and 820. It gave a positive Molisch's test, a positive ferric reaction and a positive Gibb's test. The acetate prepared by the C_5H_5N–Ac_2O method crystallized from EtOH as white needles, mp 200°. (Found: C, 60.4; H, 5.6. $C_{39}H_{40}O_{17}$ requires: C, 60.0; H, 5.3%). UV λ_{max} nm (log ε): 245 (4.15), 280 (4.59), 295 (4.56) and 320 (4.27); IR ν_{max}^{KBr} cm^{-1}: 1705, 1608, 1520, 1400, 1265 and 830; ^{1}H NMR, 90 MHz ($CDCl_3$): δ 1.27 and 1.30 (3H each, *s*, gem dimethyl), 2.00 (6H, *s*, 2 × —OCOMe), 2.04 (3H, *s*, —OCOMe), 2.31 and 2.42 (3H each, *s*, 2 × —OCOMe), 3.32 (2H, *m*, Ar—C<u>H</u>$_2$—CH<), 3.58–4.13 (3H, *m*, sugar protons), 4.71 (1H, *d*, *J* = 8 Hz, anomeric H), 4.90–5.22 (4H, *m*, 3 sugar protons and Ar—CH$_2$—C<u>H</u><), 6.19 (1H, *d*, *J* = 10 Hz, H-6), 6.40 (1H, *d*, *J* = 16 Hz, H_A), 7.00 (1H, *s*, H-4), 7.10 (2H, *d*, *J* = 8 Hz, $H_\beta H_{\beta'}$), 7.49 (2H, *d*, *J* = 8 Hz, $H_\alpha H_{\alpha'}$), 7.53 (1H, *d*, *J* = 10 Hz, H-5), 7.60 (1H, *d*, *J* = 10 Hz, H_B).

1 (50 mg) was hydrolysed with H_2SO_4 (7%) for 3 hr under reflux. The soln was extracted several times with EtOAc and D-glucose was detected in the aq. soln. The EtOAc extract, on PLC (Si gel: $CHCl_3$–MeOH, 9:1), yielded rutaretin (15 mg) [1] (mmp, co-TLC, ^{1}H NMR, UV and co-IR) and *p*-coumaric acid (6 mg) (mmp, co-TLC, UV and co-IR). **1** (100 mg) was methylated with Me_2SO_4–K_2CO_3 in Me_2CO for 52 hr and the methyl ether hydrolysed with H_2SO_4 (7%). The aglycones were extracted with EtOAc, separated by PLC (Si gel: φ-Me-HCOOEt–HCOOH, 5:4:1) and identified as 9-*O*-methyl rutaretin [1] (mmp, co-TLC, ^{1}H NMR, UV, co-IR) and *p*-methoxycinnamic acid (M^+ 178) (mmp, co-TLC, ^{1}H NMR, UV and co-IR).

1 was hydrolysed with 0.1 N $Ba(OH)_2$ soln for 6 hr at room temp. The soln was carefully neutralized with dil H_2SO_4, filtered and extracted with EtOAc. Rutaretin-1′-*O*-glucoside (**2**) obtained was purified by PLC (Si gel; EtOAc–MeOH–H_2O, 100:16.5:13.5) mp >300°; UV λ_{max} nm: 290. Permethylation of **1** and **2** by Hakomori's method [4] followed by acid hydrolysis of the products gave 2,3,4-tri-*O*-methyl-D-glucopyranose and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose which were confirmed by direct comparison with authentic samples. The β-configuration of the glucose linkage was established by the hydrolysis of **2** with emulsin.

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A NEW NATURALLY OCCURRING FLAVANONE FROM *TETRAGONIA EXPANSA*

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Key Word Index—*Tetragonia expansa*; Aizoaceae; New Zealand spinach; 2,3-dihydro-7,8-dimethoxy-2-phenyl-4H-1-benzopyran-4-one; 7,8-dimethoxyflavanone.

Naturally occurring flavanoids which have a pyrogallol oxygenation pattern in the 'A' ring but no oxygenation in the 'B' ring are rare, there being only two reported examples to date. These are isolarrien (7-hydroxy-8-methoxyflavanone) and the corresponding chalcone larrien (2′,4′-dihydroxy-3′-methoxychalcone), both isolated from *Larrea nitida* [1]. We now report the occurrence of a third natural flavanoid with this unusual oxygenation pattern, 7,8-dimethoxyflavanone, isolated from New Zealand spinach (*Tetragonia expansa* Murr.).

The purified compound isolated from a toluene leaf extract of *Tetragonia expansa* fluoresced pale blue in 375 nm UV light and gave λ_{max} 284 nm (ε 16100). The IR spectrum showed strong absorption peaks at 1600 cm^{-1} (aryl) and 1690 cm^{-1} (aryl carbonyl) but no absorption over 3000 cm^{-1} (hydroxyl region). In the ^1H NMR spectrum the compound exhibited signals due to two methoxy groups δ 3.85 (3H, *s*) and 3.90 (3H, *s*), an *ortho* aromatic pair 6.60 (1H, *d*, J = 8 Hz) and 7.65 (1H, *d*, J = 8 Hz), five other aromatic protons 7.18–7.54 (5H, *m*) and a partly resolved three-proton ABX system at 5.56 (1H, *q*, J = 5, 10.5 Hz) and 2.79–3.20 (2H, *m*). The molecular formula was obtained from the MS which has M^+ 284.104985 (100%, $C_{17}H_{16}O_4$ requires 284.104850) and also prominent ions at *m/e* 180.044487 (78%, $C_9H_8O_4$ requires 180.042253), *m/e* 152.046028 (100%, $C_8H_8O_3$ requires 152.047339) and *m/e* 137 (24%).

These data strongly suggest that the compound is a flavanone, the chemical shifts of the three-proton ABX system being particularly characteristic of this type of flavanoid [2]. There are clearly two methoxy groups which from MS data must be sited on ring 'A' as the major fragmentation pathway is via a retro Diels–Alder reaction giving a prominent ion at *m/e* 180. This further fragments to give ions at *m/e* 152 and 137; a pattern agreeing very closely with that published for other flavanones [3]. The position of the methoxy groups on ring 'A' remains to be established but they must be substituted at positions which provide for a pair of aromatic protons exhibiting *ortho*-coupling in the ^1H NMR spectrum. Of the three available possibilities, the 7,8-substitution pattern is the most probable, since in this structure one proton is deshielded by the adjacent carbonyl and this would agree with the observed resonance at δ 7.65 in the present compound.

Confirmation of the proposed structure was by synthesis. Base-catalysed condensation of 2-hydroxy-3,4-dimethoxyacetophenone with benzaldehyde afforded a chalcone which was not isolated but cyclized under acid conditions to yield 7,8-dimethoxyflavanone. Comparison of the IR, UV and ^1H NMR spectra of the synthesized 7,8-dimethoxyflavanone and the isolated natural product showed the compounds to be identical. This was confirmed by TLC and GLC.

EXPERIMENTAL

UV spectra were recorded in EtOH, IR spectra in $CHCl_3$ and ^1H NMR spectra (100 MHz) in $CDCl_3$ using TMS internal standard. MS (70 eV) were determined using a direct insertion probe. Merck precoated plates Si gel 60 F254 were used for TLC.

Isolation. Leaves of New Zealand spinach (*Tetragonia expansa*) (5.5 kg fr. wt) were extracted in toluene (12 l. × 2) for 7 days. The combined extracts were reduced to 100 ml and waxes removed by precipitation with Me_2CO and filtration. The filtrate was evapd and the residue chromatographed on a PVP column eluted with toluene plus $CHCl_3$ (0–100%). The first fraction (fluorescent blue-green in 375 nm UV) was collected, evapd, the residue dissolved in toluene (30 ml) and more wax removed by Me_2CO precipitation and filtration. The wax-free fraction was evapd and the residue dissolved in toluene (50 ml) and applied to a Si gel (Merck kieselgel 40 Art. 10180 70–230 mesh ASTM) column, which was washed with $CHCl_3$ (2.5 l.) before elution with $CHCl_3$–EtOH (19:1). The flavanone band

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